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Identification and Synthesis of New Ferulic Acid Dehydrodimers Present in Grass Cell Walls

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Seven isomeric dehydrodimers of ferulic acid (4-hydroxy-3-methoxycinnamic acid) have been synthesized and identified in extracts of saponified cell walls of cocksfoot, switchgrass, and suspension-cultured corn. Dehydrodimers (*E,E*)-4,4′-dihydroxy-5,5′-dimethoxy-3,3′-bicinnamic acid, *trans*-5-[(*E*)-2-carboxyvinyl]-2-(4-hydroxy-3-methoxyphenyl)-7-methoxy-2,3-dihydrobenzofuran-3-carboxylic acid, (*Z*)-β-{4-[(*E*)-2-carboxyvinyl]-2-methoxyphenoxy}-4-hydroxy-3-methoxycinnamic acid, (*E*)-3-{4-[(*E*)-2-carboxyvinyl]-2-methoxyphenoxy}-4-hydroxy-5-methoxycinnamic acid, (*E,E*)-4,4′-dihydroxy-3,5′-dimethoxy-β,3′-bicinnamic acid, 4,4′-dihydroxy-3,3′-dimethoxy-β,β′-bicinnamic acid, and *trans*-7-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-6-methoxy-1,2-dihydronaphthalene-2,3-dicarboxylic acid, all arise from oxidative coupling of ferulate esters in cell walls and represent products of 8–5, 8–8, 8–O–4, 4–O–5, and 5–5 radical coupling. Prior literature has acknowledged only the presence of the 5–5-coupled dehydrodimer (*E,E*)-4,4′-dihydroxy-5,5′-dimethoxy-3,3′-bicinnamic acid. Consequently, by measuring only a single dehydrodimer and assuming inappropriate response factors, ferulate dehydrodimers have been underestimated by factors of up to 20. Synthetic routes to all seven isomers have been developed to provide structural authentication and determination of GC response factors.

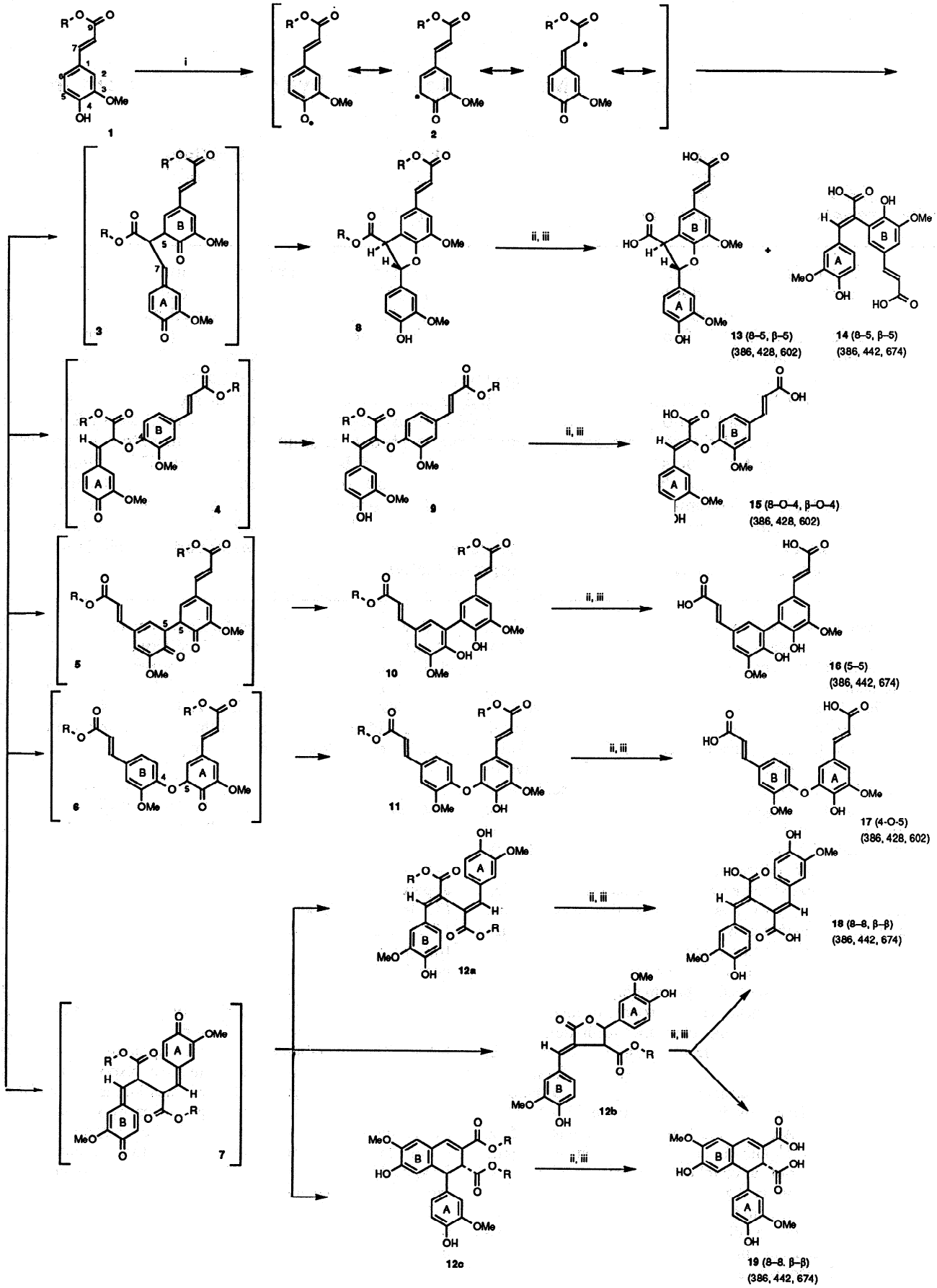
Ferulic acid is esterified to grass cell wall polysaccharides, notably to arabinoxylans at the C-5 position of α-L-arabinofuranoside moieties as has been reviewed.[1–5] Dimerization of such ferulate esters provides a pathway for cross-linking polysaccharide chains. Two disparate mechanisms have been described; photochemically induced [2 + 2]-cyclodimerization, suggested to be the predominant mechanism,[6–9] and oxidative coupling *via* the action of peroxidases to produce dehydrodimers.[10,11] The only *established* dehydrodiferulic acid, released in small amounts from grass cell walls by saponification, is the 5–5-coupled isomer **16** (Scheme 1), commonly referred to as 'diferulic acid'.[12–20] Ishii's isolation[20] of 5,5′-di-*O*-(diferul-9,9′-dioyl)-[α-L-arabinofuranosyl-(1 ⟶ 3)-*O*-β-D-xylopyranosyl-(1 ⟶ 4)-D-xylopyranose] from Driselase degradation of bamboo shoot cell walls is proof that this dehydrodimer acts as a polysaccharide cross-linking agent. The 5–5-coupled dehydrodimer was reportedly the only one produced from peroxidase/H_2O_2-mediated oxidative coupling of ferulic acid moieties synthetically esterified to guaran polysaccharide.[10] We have been unsuccessful, using either enzymic or metallic one-electron oxidations, in eliciting substantial formation of 5–5-coupled dehydrodimers from ferulate esters. For example, oxidation of compound **1** (R = methyl α-L-arabinofuranoside, FA-Ara), a model for ferulate in feruloylated arabinoxylans,[3,21,22] with silver(I) oxide (Ag_2O) gave the 2,3-dihydrobenzofuran **8** (R = Ara, Scheme 1) from 8–5 coupling in 55% yield after isolation.[23] This finding was consistent with many observations that radical-coupling products involving the 8-position (β-position in lignin nomenclature), particularly 8–5-coupling products, invariably predominate among compounds formed by oxidative dimerization of (*E*)-4-(prop-1-enyl)-phenol derivatives, including (*E*)-isoeugenol, (*E*)-coniferyl and (*E*)-*p*-coumaryl alcohols, and their aldehyde, acid or ester counterparts, by a variety of methods.[24–33] When FA-Ara was co-polymerized with coniferyl alcohol into a synthetic lignin polymer, the ferulate moiety was incorporated in 2,3-dihydrobenzofuran (8–5), alkyl aryl ether (8–O–4), and furofuran (8–8) structures.[34]

Basic synthetic studies on ferulate ester oxidative dimerization led us to speculate that dehydrodiferulate esters (structures **8**, **9**, **11** and **12** in Scheme 1) should be present in cell walls in greater amounts than the 5–5-coupled dehydrodiferulate **10**.[33] Identification, characterization, and synthesis of the major ferulic acid dehydrodimers and their analysis in plant cell wall hydrolysates are discussed in this paper.

Results and Discussion

Dehydrodiferulic Acids from Plant Materials.—Compounds **13–16**, **18** and **19** (Scheme 1), dehydrodimers arising from 8–5-, 8–O–4-, 5–5-, and 8–8-coupling processes, were readily apparent in extracts from several saponified plant materials (Table 1, Figs. 1 and 2). The 4–O–5-coupled dehydrodimer **17** may also be present in some samples at low concentrations (Table 1): the retention time of a small peak occasionally observed matches that of compound **17**, but authentication by mass spectrometry was not possible due to its low abundance. Establishment of peak identities was *via* independent synthesis of all isomers **13–19** as described below. The availability of these compounds in pure form also allowed determination of relative response factors in gas–liquid chromatography, flame ionization detection (GLC–FID) and recovery factors through the entire saponification–extraction–silylation procedure. These response factors (Table 1) were essential for valid quantitation due to their inexplicably low values relative to *p*-hydroxycinnamate monomers and the internal standard.

Isomers **13–19** have the same molecular mass but silylation for GLC and GLC–MS produced two separate sets of isomers or classes of these compounds. The first set, originally with one phenolic hydroxy and two acid groups (compounds **13**, **15** and **17**), have a nominal molecular mass of 602 (Scheme 1, Table 1); the second, with two phenolic hydroxy and two acid groups (compounds **14**, **16**, **18** and **19**) have a nominal molecular mass of 674. Mass spectra of methyl derivatives were significantly more diagnostic than those of silylated derivatives, which are



Scheme 1 The general chemistry of dehydrodimer formation and saponification. Dimerization of ferulate esters *via* phenoxy radical **2** gives intermediates **3–7** which react in the cell wall to form dehydrodiferulate esters **8–12**. During chemical analysis esters **8–12** are saponified to dehydrodiferulic acids **13–19**. The values in parentheses below the structures represent the nominal masses for the parent compound, the fully methylated, and the fully trimethylsilylated derivatives, respectively. The structure-numbering system used is based on the numbering of the monomers so as to maintain consistency; actual compound names are given in the Experimental section. *Reagents and conditions:* i, peroxidase, H_2O_2; ii, NaOH, 25 °C; iii, HCl.

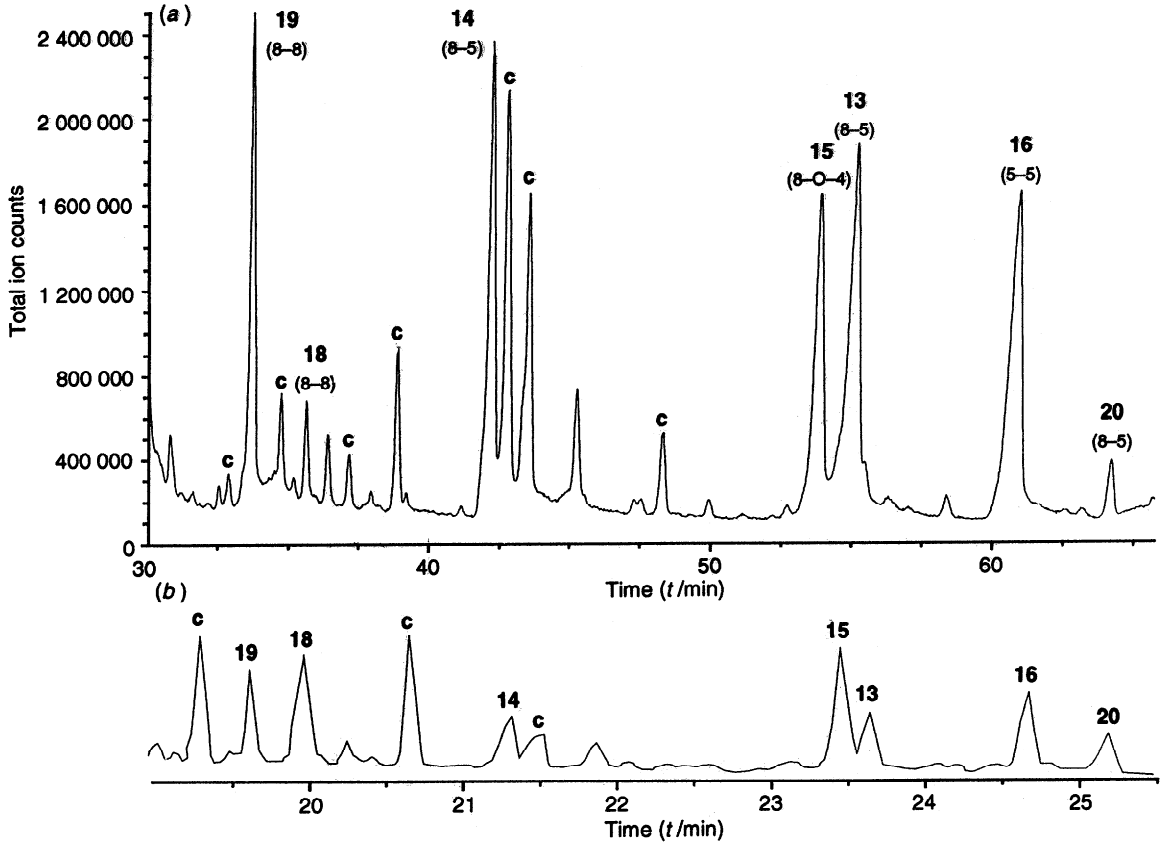


Fig. 1 GLC of dimer regions of saponified cell wall extracts. (A) Total ion chromatogram from GLC–MS of the dimers region of a saponified extract of primary cell walls from suspension-cultured corn showing dehydrodiferulic acids **13–19** (with the exception of compound **17**) and decarboxylated derivative **20**. Peaks labelled c are assigned, without further authentication, to cyclodimers based on their mass spectra. (B) Trace from a shorter GLC/FID run of switchgrass parenchyma dehydrodimers—the relative retention times vary slightly compared with the trace in A due to the temperature program used. Amounts determined from GLC are given in Table 1.

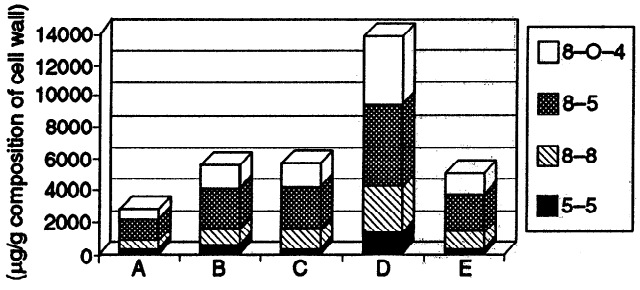
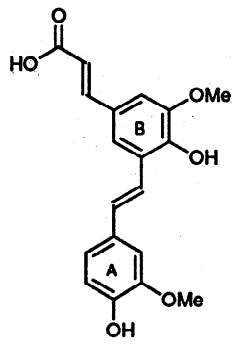


Fig. 2 Composition (μg/g of cell wall material) of dehydrodimers derived from 5–5, 8–8, 8–5, and 8–O–4 coupling modes for various plant cell wall samples: (A) suspension-cultured corn, (B) cocksfoot parenchyma, (C) cocksfoot sclerenchyma, (D) switchgrass parenchyma, (E) switchgrass sclerenchyma

largely dominated by the uninformative trimethylsilyl cation, *m/z* 73. Peaks observed from the methylated compounds were consistent with the above structure assignments but their response (relative to 2-hydroxycinnamic acid as internal standard) was extremely low so this derivatization method was not routinely used for quantitation of the dehydrodimers.

The amounts of dimers **13** + **14** + **20** (8–5), **15** (8–O–4), and **18** + **19** (8–8) match or exceed those of the only previously reported dimer, the 5–5-coupled dehydrodimer **16** (Table 1, Fig. 2). Compound **20** is a decarboxylation product from compound **14** (see Experimental section). It is surprising that these compounds have not been reported or identified previously although the percentage of each can vary substantially. From the plant materials surveyed (Table 1, Fig. 2), the amount of total ferulate dehydrodimers was seven- to twenty-fold higher than that of compound **16** alone and comprised 18–73% of the total ferulate esters in cell walls. They were lowest in undifferentiated primary walls from suspension-cultured maize and greatest in walls isolated from fully differentiated parenchyma tissues, indicating that arabinoxylans become extensively cross-linked by dehydrodimers during wall development.



20

While the photochemically derived cyclodimers of ferulic acid (truxillic and truxinic acids)[6,35,36] also contribute to the ferulic acid content of the cell wall (Fig. 1), no effort was made to identify or quantitate these cyclodimers. In plant materials

Table 1 GLC properties and data for the monomers *p*-coumaric acid (*p*CA) and ferulic acid (FA), and the dehydrodiferulic acid dimers **13–19**, and decarboxylated derivative **20**[a]

	*p*CA	FA	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	DHDFAs/**16**[e]
Relative molecular mass (MM_r)	164.16	194.19	386.36	386.36	386.36	386.36	386.36	386.36	386.36	342.35	
Methylated MM_r	192.21	222.24	428.44	442.47	428.44	442.47	428.44	442.47	442.47	384.43	
Silylated MM_r	308.52	338.55	602.90	675.09	602.90	675.09	602.90	675.09	675.09	558.89	
RF (2-hydroxycinnamic acid)[b]	1.01	0.88	0.10	0.28	0.12	0.22	n.d.	0.37	0.19	0.15	
RRT (2-hydroxycinnamic acid)[c]	1.16	1.36	2.91	2.62	2.88	3.04	3.20	2.45	2.41	3.11	
RRT (5,5-dehydrodiferulic acid, **16**)[d]	0.38	0.45	0.96	0.86	0.95	1.00	1.05	0.81	0.79	1.02	
Suspension cultured corn	380	13 470	870	350	540	410		100	470	170	7.2
Cocksfoot											
Parenchyma	510	2 170	1 460	510	1 520	530		460	650	620	10.8
Sclerenchyma	3 910	4 960	640	690	1 540	300	tr[f]	680	670	1 340	19.6
Switchgrass											
Parenchyma	4 200	7 050	3 180	860	4 230	1 370	tr	1 340	1 680	1 110	10.0
Sclerenchyma	7 800	7 000	800	680	1 430	290	tr	220	1 030	780	18.2

[a] Determinations were made in duplicate with standard deviations of ~ ± 5% of sample means for *p*CA and FA, but ~ ± 20% for dehydrodimers—see notes *b* and *c*. GLC response factors were used (see note *b*); response factors through the entire procedure were similar. [b] Response factor, relative to 2-hydroxycinnamic acid added as internal standard, through silylation and GLC. Standard deviations from 4 independent determinations were ~ ± 10% of response factors for both monomers and dimers. n.d. = not determined. [c] GLC retention time relative to 2-hydroxycinnamic acid as internal standard. [d] GLC retention time relative to the 5,5-coupled dehydrodiferulic acid **16**. [e] Ratio of the total dehydrodiferulic acid derived peaks **13–20** to the 5,5-dimer **16**. [f] Trace.

examined in this study, dehydrodimers predominate over the truxillic dimers. This may not be the case for all plant samples, but claims of dominance of [2 + 2]-cyclodimers over dehydrodimers are based on quantitation of only one dehydrodimer, **16**, and not the whole range of dimers **13–20**. Earlier papers also ignored differing GLC response factors. Hartley and Morrison[9] based their quantitation of cyclodimers on sinapic acid as internal standard, assuming a response factor of unity. The truxillic acid dimer from *p*-coumaric acid[37] has a reproducible response factor of 0.65 relative to our internal standard, 2-hydroxycinnamic acid, chosen because it does not occur in plant extracts. We suspect that cyclodimers may have been underestimated relative to the monomers due to response factor differences. Response factors for the ferulic acid dehydrodimers are even lower, ranging from 0.10 to 0.37 (Table 1), so the relative areas of dehydrodimer peaks *vs.* the *p*-coumaric acid cyclodimer are underestimated by a factor of ~ 1.75–6.5. Thus, previous measurements of the 5–5-coupled dehydrodimer **16** *vs.* the *p*-coumaric acid cyclodimer seem to be too low by a factor of almost 3 (0.65/0.22). Since compound **16** represents only a small fraction of the total dehydrodimers, the relative abundance of cyclodimers and dehydrodimers has been further distorted. Future comparisons of the relative contributions of monomers, cyclodimers and dehydrodimers must include the whole suite of dehydrodimers and address the response factors for the various compounds.

Analogous dehydrodimers of *p*-coumaric acid or mixed ferulic acid/*p*-coumaric acid dehydrodimers were not found in our preliminary screenings. The reason may be in part due to low esterification of *p*-coumaric acid to wall polysaccharides[38] and its being more highly associated with lignin.[2,3,39]

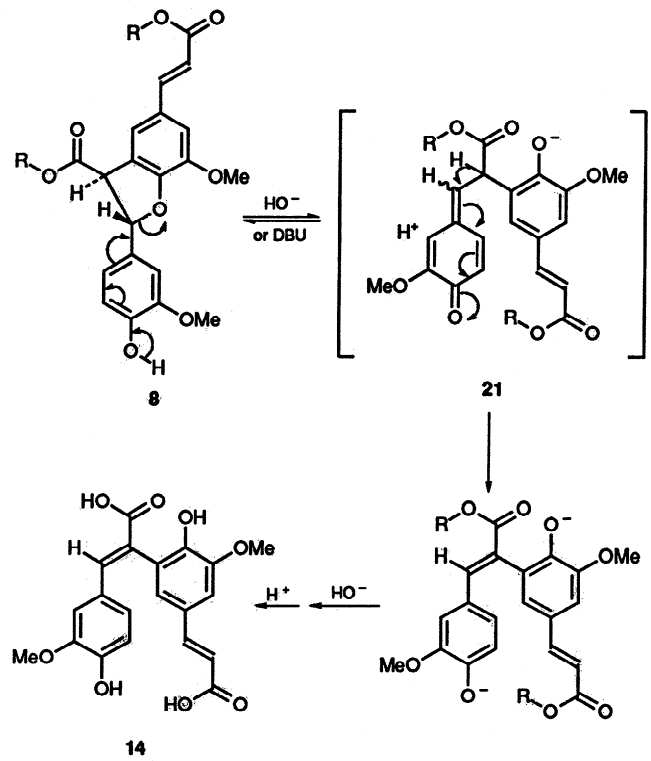
Origin of Ferulic Acid Dehydrodimers.—Electron-delocalized phenoxy radical intermediates **2**, the three most important mesomeric forms of which are shown in Scheme 1, couple 8–5, 8–O–4, 5–5, 4–O–5, and 8–8 to form dehydrodimers **8–12** *via* intermediates **3–7**. Mechanisms are analogous to those in the coupling of *p*-hydroxycinnamyl alcohols to form lignin dimers[33,40] or the cross-coupling of ferulates with coniferyl alcohol and its oligomers.[23,34] Dimers **8–12** release dehydrodiferulic acid isomers **13–19** on saponification (Scheme 1). Significant aspects of these coupling, saponification, and silylation (for GLC or GLC–MS) reactions are described in the following sections. Implications for cell-wall development and mechanistic details are more fully discussed in Quideau's thesis.[33]

8–5 *Coupling Products.*—The 8–5-coupled dehydrodimer **8** is formed from the quinone methide intermediate **3** *via* deprotonation/aromatization of the B-ring and intramolecular phenolate attack on the quinone method (Scheme 1). Cyclization is faster than elimination of the acidic 8-proton (α to its ester carbonyl group), which could also re-aromatize the A-ring; no acyclic dimer **14** was detected by GLC analysis of the saponified product mixture from Ag_2O oxidation of ethyl (*E*)-ferulate from which the 2,3-dihydrobenzofuran (or phenylcoumaran) **8** (R = Et) had been chromatographically removed prior to saponification. Analogous dihydrobenzofurans have been previously identified from oxidative cross-coupling between a ferulate moiety (8-position) and coniferyl alcohol oligomers (5-position) in a synthetic co-polymer.[34] Oxidation of (*E*)-coniferaldehyde formed the bisformyl analogue of the stilbene **14**, as the sole 8–5-coupling product;[26] the stronger electron-withdrawing aldehydic carbonyl favoured 8-proton elimination over B-ring deprotonation/aromatization and intramolecular nucleophilic attack.

Saponification of diester **8** to give diacid **13** competes with opening of the 2,3-dihydrobenzofuran ring to a quinone methide intermediate **21** (Scheme 2),[41] and subsequent elimination of the 8-proton (3-H of the 2,3-dihydrobenzofuran—see caption to Scheme 1 for numbering conventions) producing the stilbene **14**. Treatment of the free acid **13** with base at room temperature does not produce compound **14** because the 8-proton, α to a carboxylic acid, is not as acidic as the 8-proton in diester **8**. Saponification of ester models **8** (R = Me, Et, Ara) results in varying amounts of the cyclic and acyclic products **13** and **14**. The silylation conditions used [*N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) + pyridine] convert diacid **13** into a mixture of silyl derivatives of both diacids **13** and **14**. Determination by GLC of the amount of 8–5-coupled dehydrodimers should therefore include the peaks corresponding to the silyl derivatives of both diacids **13** and **14** as well as compound **20**.

8–O–4 *Coupling Products.*—The 8–O–4 coupled dimer **9** is produced in unsaturated form following oxidative dimerization. The acidic 8-proton of the quinone methide intermediate **4** eliminates precluding intermolecular nucleophilic attack on the quinone method (such as is seen in *p*-hydroxycinnamyl alcohol dimerizations) and results in a single isomer, (*Z*)-**9**. This has been previously observed for both (*E*)-coniferaldehyde dimerization,[26] and in the 8–O–4 cross-coupling between a ferulate ester and coniferyl alcohol oligomers.[23,34] Saponification cleanly yields diacid **15** as a single isomer (Scheme 1).

5–5 *and* 4–O–5 *Coupling Products.*—The 5–5- and 4–O–5-coupled dehydrodimers **10** and **11** are produced from their intermediates **5** and **6** *via* deprotonation/aromatization (Scheme 1). No 5–5- or 4–O–5-coupled dehydrodimers were isolated from oxidative coupling of isoeugenol,[27] coniferyl alcohol,[32,42,43] *p*-coumaryl alcohol,[28] or coniferaldehyde,[26] affirming that oxidative coupling of 4-(prop-1-enyl)phenols always involves the predominant participation of the 8-radical mesomer.[33] Although no 5–5 dehydrodimer was obtained from Ag_2O oxidation of ethyl (*E*)-ferulate,[44] oxidation of FA-Ara



Scheme 2 Mechanism for the formation of the stilbene **14** from base treatment of the 8–5-coupled dehydrodiferulate ester **8** in the cell wall or its synthetic preparation from compound **8** (R = Et) using DBU followed by saponification

gave detectable amounts,[44] implying that substituent effects, as well as solvent effects[32] and presumably matrix effects, influence the course of dimerization to some extent.

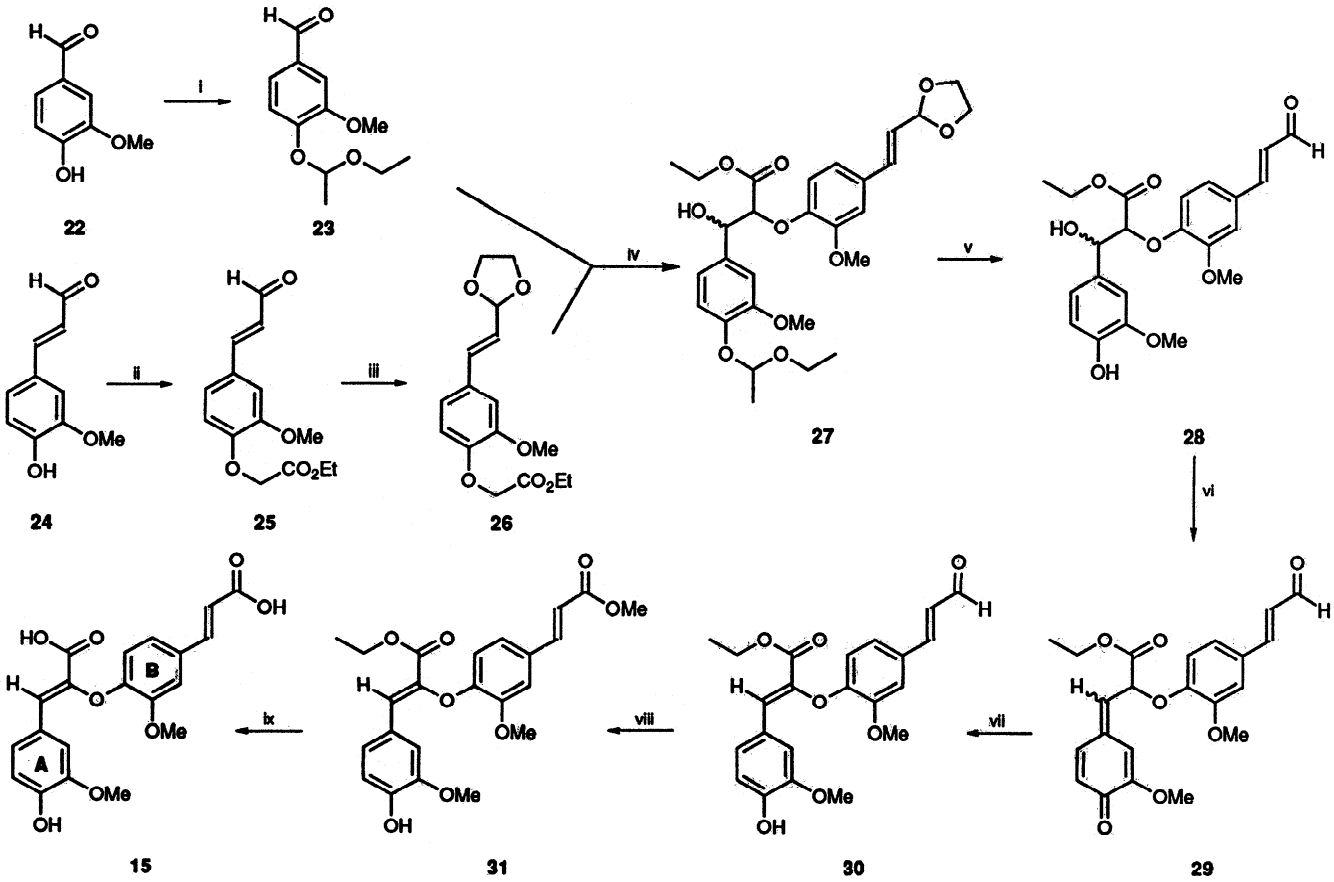
8–8 *Coupling Products.*—The 8–8 oxidative coupling pathway initially gives rise to the bis(quinone methide) **7** (Scheme 1). As observed for quinone methide **4**, aromatization through 8-proton elimination is also effective from dimer **7**. Elimination of both 8-protons to give the bis(benzylidene)succinate **12a** is not exclusive and other pathways appear to lead to compounds **12b** and/or **12c**. Dimers **12a** and **12c** are interesting consequences of 8–8 coupling, since both are potential polysaccharide cross-linking structures. They release compounds **18** and **19** upon saponification (Scheme 1). If the furanone **12b** is formed in the cell wall *via* initial aromatization of one quinone methide moiety of intermediate **7** by 8-proton elimination followed by addition of water to the second quinone methide and transesterification, its parent acid released by saponification can cyclize to compound **19** upon acidification. Alternatively, opening of the γ-lactone ring, 8-proton elimination from the quinone methide intermediate, and saponification would produce dimer **18** (see Experimental section). Analogues of the furanone **12b** have been isolated from oxidative coupling of various 4-(prop-1-enyl)phenol derivatives.[45–48] One polysaccharide component is released, so compound **12b** is not considered to be a polysaccharide cross-linking structure.

Silylation also results in conversion of the furanone **12b** (R = H) into the silyl derivative of compound **18**, since its silyl ester **12b** (R = Me_3Si) can undergo a pyridine-mediated 8-proton elimination, as noted above for the dihydrobenzofuran **13**. Hence, the total contribution of 8–8-coupled dehydrodimers must include silylated forms of both compounds **18** and **19**. Owing to these complicating acid and base rearrangements, the exact contribution of 8–8-coupled dehydrodimers to polysaccharide cross-linking remains unknown. Future work will be aimed at determining the stereochemistry of the 8–8 coupling mode and the 8–8-coupled structures in the cell wall.

Synthesis of Dehydrodiferulic Acid Isomers.—Compound **16** was obtained from the Pew collection currently held by the U.S. Forest Products Laboratory; it was fully authenticated by NMR and high-resolution mass spectrometry (see Experimental section).

The 2,3-dihydrobenzofuran **8** (R = Et) was synthesized in ~30% isolated yield from ethyl (*E*)-ferulate using Ag_2O oxidation. Previously we have obtained isolated yields of 55% using FA–Ara as substrate.[23] Treatment of diester **8** (R = Et) with 2 mol dm^{-3} sodium hydroxide at room temperature for 20 h produced the diacid stilbene (*E,E*)-**14** *via* the intermediate quinone methide **21** (Scheme 2) and the diacid 2,3-dihydrobenzofuran (*E*)-**13**, along with a small amount of the stilbene **20** formed by decarboxylation of diacid **14**. The production of both the cyclic (2,3-dihydrobenzofuran) and acyclic diacids **13** and **14** results from apparently similar rates of ester hydrolysis and 8-proton elimination from the quinone methide **21**. Alternatively, compound **14** was more efficiently prepared by the action of the strong non-nucleophilic base 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU)[23] on the dihydrobenzofuran **8** (R = Et), followed by saponification (Scheme 2).

Early attempts to make compound **15** by a variety of oxidative schemes, including the use of chromium(VI), hypochlorite, and Swern reagents, from methyl 4-*O*-[2-(4-hydroxy-3-methoxyphenyl)-1-hydroxymethyl-2-oxoethyl]ferulate,[49] or by ethoxycarbonylation of methyl 4-*O*-[2-(4-hydroxy-3-methoxyphenyl)-2-oxoethyl]ferulate[49] met with failure. A synthesis (Scheme 3) was achieved based on that of 4-*O*-[2-hydroxy-2-(4-

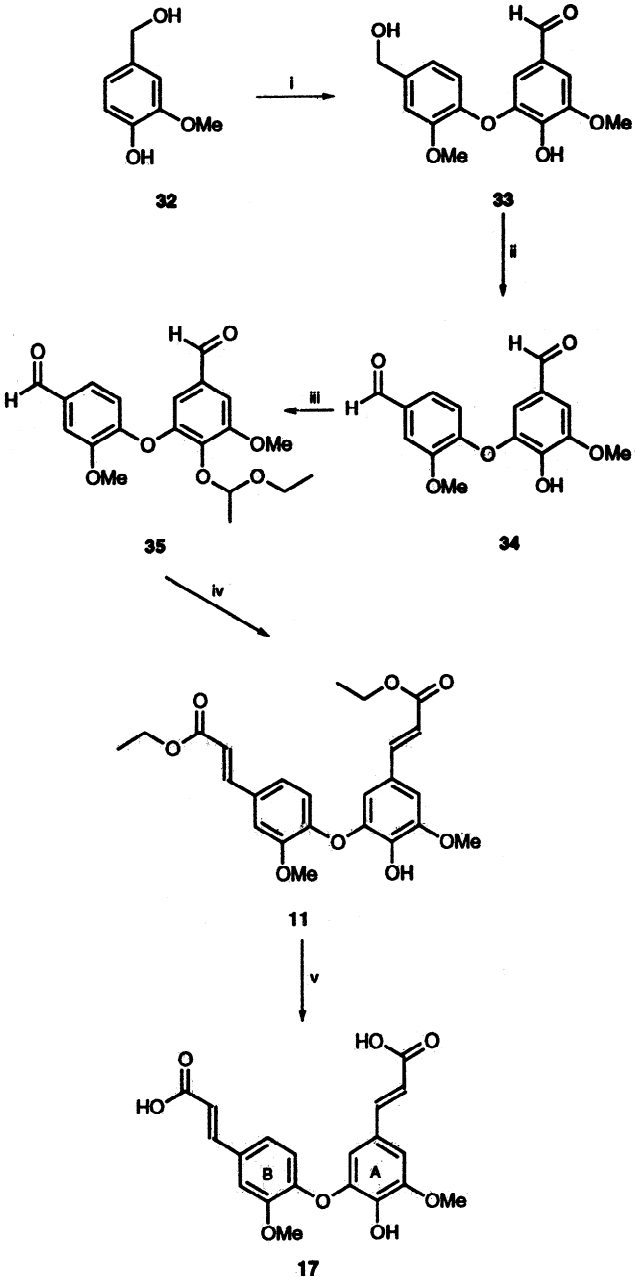


Scheme 3 Scheme for synthesis of the 8–O–4-coupled dehydrodiferulic acid **15**. *Reagents and conditions:* i, Ethyl vinyl ether, pyridinium toluene-*p*-sulfonate, CH_2Cl_2; ii, ethyl chloroacetate, K_2CO_3/KI, acetone; iii, ethylene glycol, toluene-*p*-sulfonic acid, benzene; iv, LDA, THF, −78 °C; v, 1 mol dm^{-3} HCl, THF; vi, Me_3SiBr, CH_2Cl_2; then DBU; vii, DBU, CH_2Cl_2; viii, NaCN, MnO_2, MeOH–CH_2Cl_2; ix, 2 mol dm^{-3} NaOH, 1,4-dioxane.

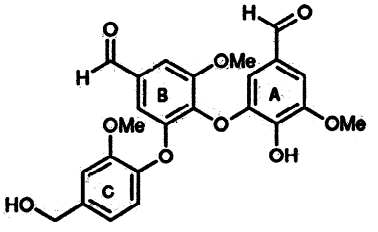
hydroxy-3-methoxyphenyl)-1-(hydroxymethyl)ethyl]-3-methoxycinnamaldehyde (guaiacylglycerol-β-coniferaldehyde ether):[33,50] compound **27** was deprotected to the aldehyde **28** and the corresponding benzyl bromide (Br replaces OH) was generated by using trimethylsilyl bromide;[51] DBU generated the quinone methide **29** *in situ* and subsequent 8-proton elimination gave a single isomer of the styryl ether **30**. Generation of a single isomer, the (*Z*)-isomer, has been observed in reactions involving 8-proton elimination from analogous quinone methides.[23] Conversion of the aldehyde **30** into the methyl ester **31** was accomplished by Corey–Gilman–Ganem oxidation.[52] Saponification gave (*Z*,*E*)-8–O–4-coupled dehydrodiferulic acid **15**. Stereochemical nomenclature introduces some confusion when comparing compounds **14** (above) and **15** due to the *E*/*Z* convention.[53] In both compounds **14** and **15**, the acid moiety is *E* to ring A, giving rise to an (*E*)-isomer for **14**, but a (*Z*)-isomer for compound **15** because the 8-aryloxy substituent has a higher Cahn–Ingold–Prelog priority than does the acid group.[54]

Literature routes to 4–O–5 compounds are lengthy.[55] Our convenient route, Scheme 4, to the 4–O–5-coupled dehydrodiferulic acid **17**, is based on Ag_2O oxidation. Ag_2O-mediated oxidation of vanillin **22** led to a complex mixture of oligomeric/polymeric materials. We suspected that vanillyl alcohol **32**, a less conjugated structure than vanillin, might undergo some 4–O–5 coupling. Reaction of vanillyl alcohol **32** with 1.5 mol equiv. of Ag_2O in acetone (Scheme 4) produced the 4–O–5-coupled product **33**, in which one benzyl alcohol group had been oxidized to the aldehyde, as the main dehydrodimer, in up to 30% yield (see Experimental section). TLC monitoring indicated that vanillin was produced early in the reaction, suggesting selective coupling between vanillyl alcohol at the 4–O-position and vanillin at the 5-position. A small amount of the bis-4–O–5-coupled trimer **36** was also isolated, indicating that the 4–O–5 coupling mode is favoured under these conditions. No 5–5-coupled product was isolated. Oxidation of the benzyl alcohol **33** with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) gave the 4–O–5-coupled dehydrodivanillin **34**. Protection, completion of the two carboxyvinyl side-chains by a Wittig–Horner reaction, deprotection, and saponification gave the (*E*,*E*)-4–O–5-coupled dehydrodiferulic acid **17** (Scheme 4).

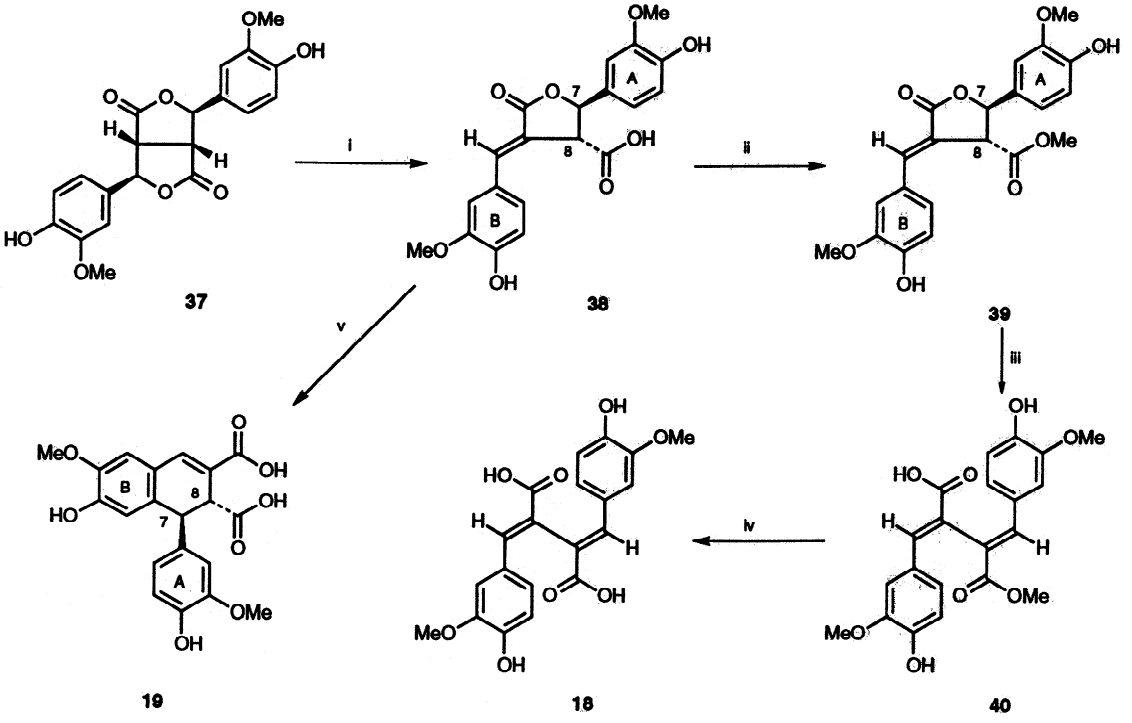
The lignans **18** and **19** were both derived from the dehydrodiferulic acid dilactone 4-*cis*,8-*cis*-bis-(4-hydroxy-3-methoxyphenyl)-3,7-dioxabicyclo[3.3.0]octane-2,6-dione **37**[56,57] (Scheme 5). Alkali treatment of bis-lactone **37** gave the γ-lactone *trans*-**38** (**12b**, R = H) upon acidification. Selective methylation of the carboxylic group of compound **38** with diazo(trimethylsilyl)methane[58] gave ester *trans*-**39**. The *trans*-orientations of the A7 and A8 protons in lactones **38** and **39** were deduced from their small coupling constants (2.8–3.0 Hz) and a comparison with literature data of the methyl sinapate analogue of compound **39**.[45] The γ-lactone **39** was well suited for generation of a bis-α,β-enone system: DBU-mediated elimination of the 8-proton afforded the monomethyl bis-(benzylidene)succinate derivative **40**, whose saponification gave the 8–8-coupled dehydrodiferulic acid **18**, presumably as the (*E*,*E*)-isomer. This lignan had previously been obtained in moderate yield as its dimethyl ether dimethyl ester from MeI–KF–dimethylformamide (DMF) treatment of bis-lactone **37**.[59] Synthesis of the dimethyl ester of the naphthalenedicarboxylic acid **19** by treatment of bis-lactone **37** with methanolic HCl and of thomasidioic acid by HCl treatment of the corresponding dehydrodisinapic acid dilactone have been reported.[56,60] Similar acid-catalysed rearrangements of our bis-lactone **37** repeatedly led to product mixtures in which the γ-lactones **38** or **39** were recognized as intermediates by TLC and NMR spectroscopy. The γ-lactone *trans*-**38**, made from bis-lactone **37**



Scheme 4 Scheme for preparation of the 4–O–5-coupled dehydrodiferulic acid **17**. *Reagents:* i, Ag_2O, acetone; ii, DDQ, THF; iii, ethyl vinyl ether, pyridinium toluene-*p*-sulfonate, CH_2Cl_2; iv, NaH, THF; then triethyl phosphonoacetate, THF; then HCl, THF; v, 2 mol dm^{-3} NaOH, 1,4-dioxane.



as stated above, rearranged to the desired 1-aryl-*trans*-1,2-dihydronaphthalene derivative **19** on being refluxed with aq. HCl in 1,4-dioxane. The *trans* diaxial orientation of the aryl group at C-1 (A7 in our numbering scheme, Scheme 1) and



Scheme 5 Synthesis of the bis(benzylidene)succinic acid and aryldihydronaphthalene lignans **18** and **19** from the furofuranoid lignan **37**. *Reagents:* i, 2 mol dm^{-3}; NaOH; ii, Me_3SiCHN_2, MeOH–hexanes; iii, DBU, CH_2Cl_2; iv, 40% KOH; v, 1 mol dm^{-3} HCl, 1,4-dioxane.

the carboxy group at C-2 (or A8) in diacid **19** was deduced from the small coupling constant between protons A7 and A8 (1.8 Hz) and comparison with NMR data of thomasidioic acid.[60–62]

Conclusions.—Ferulic acid dehydrodimers have the potential to cross-link polysaccharides to lignin in the cell wall, perhaps providing a template for lignification,[3,5,63] and may be important in modifying mechanical properties of the plant cell wall.[11,16–18] Studies with maize cell walls suggest that extensive ferulate dehydrodimer formation occurs at the onset of lignification[64] when generation of hydrogen peroxide is initiated to stimulate peroxidase-mediated coupling of *p*-hydroxycinnamyl alcohols.[65] The release of the entire series of ferulic acid dehydrodimers by saponification of grass cell walls is proof that their arabinoxylan ester parents are formed by oxidative coupling. These ferulate ester dehydrodimers are likely to undergo further oxidative coupling with *p*-hydroxycinnamyl alcohol monomers or oligomers during lignification to form bridges between cross-linked polysaccharide chains and lignin, not only as α-ether structures derived from lignin quinone methide intermediates as recently suggested for the 5–5-coupled isomer,[66] but also as several C–C- and C–O-linked coupling structures.[3,34,64] Such processes will clearly reduce the yields of dehydrodimers released by saponification, contributing further to their underestimation.

Underestimation of ferulic acid dehydrodimers by analysis and quantitation of only the 5–5-coupled isomer is a serious shortcoming in past compositional work. The occurrence of other dehydrodimers in grasses parallels the ready formation of non-5–5-coupled dehydrodimers in the laboratory and provides a compelling endorsement of model studies. Past research has focused on the 5–5-coupled dehydrodimer to the complete exclusion of the more predictable 8–5, 8–O–4, and 8–8 dehydrodimers. These other dehydrodimers *are* present in the cell walls of the grasses we have surveyed at combined levels that far exceed that of 5–5-coupled dehydrodiferulic acid and the photochemically derived cyclodimers which have been claimed[6–9] to be more prevalent. Consequently, ferulate dehydrodimers must assume a more substantial role in defining cell wall mechanical properties and in the limitations to digestibility caused by polysaccharide–polysaccharide and lignin–polysaccharide cross-linkages.

Sound analytical methodology remains a challenge to develop because of the low and somewhat variable response factors. An internal standard for dimers with an appropriate retention time and a relative response factor closer to unity should be found. Methods to minimize interconversion of some compounds during derivatization would also be valuable.

Experimental

M.p.s were measured on an Electrothermal digital m.p. apparatus and are uncorrected. Evaporations were conducted under reduced pressure at temperatures less than 42 °C unless otherwise noted. Further elimination of organic solvents, as well as drying of the residues, was accomplished under high vacuum (90–120 mTorr) at room temperature. Column chromatography was performed on silica gel 60 (230–400 mesh) and TLC was performed with Alugram Sil-G/UV_{254} plates (Macherey-Nagel), with visualization by UV light.

NMR spectra of samples in [$^2H_6$]acetone (unless otherwise noted) were run at 300 K on a Bruker AMX-360 360 MHz narrow-bore instrument fitted with a 5 mm 4-nucleus (QNP) probe with normal geometry (proton coil further from the sample). *J*-Values are given in Hz. The central solvent signals were used as internal reference (^1H, δ 2.04; ^{13}C, δ_C 29.8). One- and two-dimensional NMR spectra were obtained using standard Bruker pulse programs; ^1H–^1H correlation information was obtained with a delayed COSY experiment, using a fixed delay of 350 ms, whereas ^1H–^{13}C correlation information was obtained with the usual combination of the inverse-detected one-bond and long-range ^1H–^{13}C correlation experiments, HMQC[67] and HMBC.[68] Full data for all title compounds and key intermediates recorded in [$^2H_6$]acetone, [$^2H_6$]dimethyl sulfoxide and $CDCl_3$, are given in the recently released NMR database of plant cell-wall model compounds.[69]

High-resolution EI–MS data were collected on a Kratos MS-

Table 2 ^{13}C NMR shifts of the dehydrodiferulic acids **13**–**19** and decarboxylated derivative **20**[a]

Carbon	13	14	15	16	17	18	19	20
A1	132.49	127.58	125.31	126.60	126.71	127.94	136.12	130.90
A2	110.72	113.26	113.77	109.97	108.03	113.51	111.98	110.16
A3	148.53	147.86	148.30	148.92	150.12	148.19	148.10	148.61
A4	147.81	148.98	149.46	147.38	141.39	149.25	146.06	147.61
A5	115.78	115.63	115.96	125.62	144.44	115.93	115.48	155.99
A6	120.08	126.36	126.06	126.07	114.46	125.60	120.67	121.14
A7	88.56	141.81	128.49	145.89	145.24	142.26	46.00	130.68
A8	56.22	126.34	138.28	116.28	117.00	126.15	48.08	120.61
A9	172.55	169.15	164.51	168.36	168.06	168.46	173.62	
B1	129.38	127.26	130.10		131.07		124.64	126.98
B2	113.34	110.27	112.39		112.70		113.06	109.15
B3	145.73	149.10	150.23		151.39		147.48	148.83
B4	150.96	148.01	148.90		149.34		149.29	146.96
B5	128.05	125.14	114.39		118.34		116.92	125.47
B6	118.95	125.60	122.92		122.81		132.35	120.84
B7	145.59	145.80	145.25		145.38		137.58	146.00
B8	116.75	116.23	117.50		117.95		124.34	116.50
B9	168.18	168.56	167.91		168.02		169.32	168.22
A3-OMe	56.31	55.48	55.92	56.52	56.76	56.05	56.19	56.25
B3-OMe	56.49	56.55	56.47		56.41		56.39	56.56

[a] Values were determined in [$^2H_6$]acetone at 300 K with the central solvent peak as internal reference (δ_C 29.80 ppm). The carbonyl A9/B9 chemical shifts can vary by 0.20–0.75 ppm and the side-chain signals can shift up to 0.35 ppm, presumably due to H/D exchange at the carboxylic hydroxy groups with D_2O/HDO in [$^2H_6$]acetone.

80RFA spectrometer. Percentage values in parentheses refer to the height relative to the spectrum base peak (usually *m/z* 73 in silylated samples).

Acetone and methylene dichloride (CH_2Cl_2) were dried by passage through a column of alumina. Tetrahydrofuran (THF) was distilled from sodium–benzophenone immediately before use. Diisopropylamine was distilled from sodium. Light petroleum refers to the fraction boiling in the range 40–60 °C.

Isolation of Cell Walls.—Suspension cultures of Black Mexican Sweet corn (*Zea mays*, cv. Black Mexican) were grown under conditions similar to those of Kieliszewski and Lamport.[70] Cells were suspended in ice-cold 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid (HEPES) buffer (25 mmol dm^{-3}, pH 7.5 with 25 mmol dm^{-3} 2-sulfanylethanol), ruptured with a probe-type sonicator and washed sequentially with HEPES buffer, 1% sodium dodecyl sulfate (SDS), water, and acetone to produce cell walls. Parenchyma and sclerenchyma cell walls were isolated from leaf blades of vegetative cocksfoot (orchardgrass, *Dactylis glomerata* L.) and switchgrass (*Panicum virgatum* L.).[71]

Saponification of cell walls. Cell walls (50–100 mg) were treated for 20 h at 25 °C with 2 mol dm^{-3} NaOH (4 cm^3) under N_2. 2-Hydroxycinnamic acid (0.1 mg) was added as an internal standard. Samples were acidified with 12 mol dm^{-3} HCl (0.7 cm^3) and extracted into diethyl ether (2×; 3 cm^3). Dried extracts were silylated with pyridine (15 mm^3) and BSTFA (30 mm^3) for 30 min at 60 °C. Trimethylsilylated (TMS) derivatives of phenolic acids were separated by GLC (Perkin-Elmer 8500) using a 0.25 mm × 30 m DB-1 (J & W Scientific) column and a flame-ionization detector with He as carrier gas (10 cm^3 min^{-1}). The column was held at 170 °C for 1 min, ramped at 10 °C min^{-1} to 325 °C, and held for 10 min. The injector and detector were set at 325 °C. The amounts of individual acids were calculated using response factors determined by running *p*-coumaric, ferulic, and each of the dehydrodiferulic acids through the saponification procedure in the presence of cellulose (Sigma-Cell 100, Sigma Chemical Company). Recoverability was often extremely poor without added cellulose. Response factors through the procedure were comparable to directly determined GLC response factors (Table 1) but were more variable. Even with pure authentic standards, response-factor determination was difficult in some instances due to isomerization during the silylation procedure. Silylated dimers were stable for only a few hours and were therefore analysed quickly. For mass spectrometry, silylated samples were separated by a 0.25 mm × 60 m DB-1 column on an HP 5890 gas chromatograph using He as a carrier gas and detected with an HP 5970 mass-selective detector. The column was held at 200 °C for 1 min, ramped at 10 °C min^{-1} to 325 °C, and held for 60 min. The injector and detector were set at 300 °C.

(E,E)-4,4′-*Dihydroxy*-5,5′-*dimethoxy*-3,3′-*bicinnamic Acid* (E,E)-**16** (5–5-*Coupled Dehydrodiferulic Acid*).—This compound was kindly provided from the J. C. Pew collection by the US Forest Products Laboratory, Madison, WI. Some ^{13}C NMR assignments (Table 2), authenticated here, differ from those reported in [$^2H_6$]acetone–D_2O (9:1);[72] δ_H* 3.97 (6 H, s, 2 × OMe), 6.42 (2 H, d, *J* 15.9, 8-H), 7.21 (2 H, d, *J* 2.0, 6-H), 7.35 (2 H, d, *J* 2.0, 2-H), 7.64 (2 H, d, *J* 15.9, 7-H); δ_C see Table 2. Trimethylsilyl-**16** [Found: M^+, 674.2549 (19%). $C_{32}H_{50}O_8Si_4$ requires M, 674.2583].

Synthesis of the 8–5-*Coupled Dehydrodiferulic Acids* **13**, **14** *and* **20**.—*Ethyl* trans-5-[(E)-2-*ethoxycarbonylvinyl*]-2-(4-*hydroxy*-3-*methoxyphenyl*)-7-*methoxy*-trans-2,3-*dihydrobenzofuran*-3-*carboxylate* (E)-**8** (*R* = *Et*). Ethyl (*E*)-ferulate **1** (R = Et; 1.02 g, 4.59 mmol) was dissolved in dry acetone (20 cm^3). Silver(I) oxide (1.28 g, 5.52 mmol) was added and the reaction mixture was stirred at room temperature. The acetone solution gradually became orange-reddish. The reaction was monitored by TLC [Et_2O–light petroleum (2:1)], indicating that, in addition to the formation of the desired 2,3-dihydrobenzofuran **8** (R = Et), other dehydrodimers and/or trimeric compounds were formed as evidenced by a slower moving spot, as well as some oligo/polymeric products. The mixture was stirred for typically 2–3 h, after which time the reaction mixture was filtered through Celite. The filtrate was evaporated and submitted to silica gel chromatography [Et_2O–light petroleum (2:1)] to give the diethyl 8–5-coupled dehydrodiferulate (*E*)-**8** as a solid, which was recrystallized from acetone–light

* Locants refer to the parent cinnamic acid framework.

petroleum (285 mg, 28%) as needles, m.p. 152.8–153.1 °C; δ_H* 1.27 and 1.29 (3 H each, *J* 7.1, 2 × *Me*CH_2O), 3.82 (3 H, s, A3-OMe), 3.91 (3 H, s, B3-OMe), 4.18 (2 H, q, *J* 7.1, B9-O*C*H_2Me), 4.25 (2 H, m, A9-O*C*H_2Me), 4.43 (1 H, d, *J* 8.0, A8-H), 6.03 (1 H, d, *J* 8.0, A7-H), 6.41 (1 H, d, *J* 15.9, B8-H), 6.84 (1 H, d, *J* 8.1, A5-H), 6.91 (1 H, dd, *J* 8.1 and 1.9, A6-H), 7.08 (1 H, d, *J* 1.9, A2-H), 7.27 (1 H, br s, B6-H), 7.31 (1 H, br s, B2-H), 7.62 (1 H, d, *J* 15.9, B7-H) and 7.87 (1 H, s, A4-OH); δ_C 14.48 (A9-OCH$_2$*Me*), 14.63 (B9-OCH$_2$*Me*), 56.06 (A8), 56.31 (A3-OMe), 56.49 (B3-OMe), 60.56 (B9-O*C*H$_2$Me), 62.20 (A9-O*C*H$_2$Me), 88.34 (A7), 110.74 (A2), 113.30 (B2), 115.82 (A5), 116.69 (B8), 118.91 (B6), 120.18 (A6), 127.41 (B5), 129.46 (B1), 132.10 (A1), 145.22 (B7), 145.82 (B3), 147.97 (A4), 148.56 (A3), 150.99 (B4), 167.28 (B9) and 171.10 (A9). The slower moving fraction was also collected (106 mg) and saponified prior to GLC analysis, which indicated the presence of the silyl derivatives of the 8–8-coupled dehydrodimers **18** and **19**.

(E,E)-4,4′-*Dihydroxy*-3,5′-*dimethoxy*-β,3′-*bicinnamic acid* (E,E)-**14**; trans-5-[(E)-2-*carboxyvinyl*]-2-(4-*hydroxy*-3-*methoxyphenyl*)-7-*methoxy*-2,3-*dihydrobenzofuran*-3-*carboxylic acid* (E)-**13**; *and* (E)-4-*hydroxy*-3-[(E)-4-*hydroxy*-3-*methoxystyryl*]-5-*methoxycinnamic acid* (E,E)-**20**; *base treatment of diester* **8** (*R* = *Et*). The 2,3-dihydrobenzofuran **8** (R = Et; 91 mg, 0.206 mmol) was dissolved in 1,4-dioxane (2 cm^3) and hydrolysed under N_2 with degassed 2 mol dm^{-3} NaOH (5 cm^3) at room temperature for *ca.* 20 h. The solution was acidified with 2 mol dm^{-3} HCl and partitioned between EtOAc and saturated aq. NaCl. The organic layer was dried over Na_2SO_4 and evaporated to give a yellow foam (82 mg), which was submitted to preparative TLC (PLC) [$CHCl_3$–EtOAc–AcOH (10:1:0.1), multiple elution] to afford the stilbene (*E*,*E*)-**14** (34 mg, 43%), the 2,3-dihydrobenzofuran (*E*)-**13** (22 mg, 28%) and the stilbene (*E*,*E*)-**20** (6 mg).

(E,E)-4,4′-*Dihydroxy*-3,5′-*dimethoxy*-β,3′-*bicinnamic acid* (E,E)-**14**, fine crystals, m.p. 160–165 °C (decomp.); δ_H* 3.45 (3 H, s, A3-OMe), 3.95 (3 H, s, B3-OMe), 6.38 (1 H, d, *J* 15.9, B8-H), 6.71 (1 H, d, *J* 8.2, A5-H), 6.73 (1 H, d, *J* 2.0, A2-H), 6.85 (1 H, dd, *J* 8.2 and 2.0, A6-H), 7.03 (1 H, d, *J* 1.9, B6-H), 7.37 (1 H, d, *J* 1.9, B2-H), 7.60 (1 H, d, *J* 15.9, B7-H) and 7.81 (1 H, s, A7-H); δ_C see Table 2. Trimethylsilyl-**14** [Found: M$^+$, 674.2704 (51%). $C_{32}H_{50}O_8Si_4$ requires M, 674.2583. Found: MH$^+$, 675.2680 (76%). $C_{32}H_{51}O_8Si_4$ requires M, 675.2661].

trans-5-[(E)-2-*Carboxyvinyl*]-2-(4-*hydroxy*-3-*methoxyphenyl*)-7-*methoxy*-2,3-*dihydrobenzofuran*-3-*carboxylic acid* (E)-**13**, fine crystals, m.p. 169–174 °C (decomp.) (bright red liquid at 178 °C); δ_H* 3.83 (3 H, s, A3-OMe), 3.91 (3 H, s, B3-OMe), 4.39 (1 H, d, *J* 7.8, A8-H), 6.05 (1 H, d, *J* 7.8, A7-H), 6.39 (1 H, d, *J* 15.9, B8-H), 6.83 (1 H, d, *J* 8.1, A5-H), 6.91 (1 H, dd, *J* 8.1 and 2.0, A6-H), 7.08 (1 H, d, *J* 2.0, A2-H), 7.29 (1 H, br s, B2-H), 7.33 (1 H, br s, B6-H) and 7.62 (1 H, d, *J* 15.9, B7-H); δ_C see Table 2. Trimethylsilyl-**13** [Found: M$^+$, 602.2192 (11%). $C_{29}H_{42}O_8Si_3$ requires M, 602.2188]. Some conversion into TMS-**14** occurs under silylation conditions.

(E)-4-*Hydroxy*-3-{2-[(E)-4-*hydroxy*-3-*methoxystyryl*]}-5-*methoxycinnamic acid* (E,E)-**20**, yellow oil, δ_H* 3.91 (3 H, s, A3-OMe), 3.95 (3 H, s, B3-OMe), 6.44 (1 H, d, *J* 15.9, B8-H), 6.83 (1 H, d, *J* 8.1, A5-H), 7.05 (1 H, dd, *J* 8.1 and 2.0, A6-H), 7.22 (1 H, d, *J* 2.0, A2-H), 7.23 (1 H, d, *J* 1.9, B2-H), 7.31 and 7.33 (AB qt, $\Delta\nu_{AB}$ 7.389, J_{AB} 16.5, A7- and A8-H), 7.54 (1 H, d, *J* 1.9, B6-H) and 7.63 (1 H, d, *J* 15.9, B7-H); δ_C see Table 2. Trimethylsilyl-**20** [Found: M$^+$, 558.2272 (20%). $C_{28}H_{42}O_6Si_3$ requires M, 558.2289].

Alternatively (Scheme 2), compound **14** was more readily obtained by addition of DBU (125 mm^3, 0.836 mmol) to a solution of the 2,3-dihydrobenzofuran **8** (R = Et; 83.5 mg, 0.189 mmol) in CH_2Cl_2 (2 cm^3). The yellow solution was stirred at room temperature for 4 h, after which time the resulting dark red solution was diluted in CH_2Cl_2, washed successively with 3% HCl, water and saturated aq. NaCl. The organic layer was dried (Na_2SO_4), evaporated, and the residue was submitted to silica gel chromatography [$CHCl_3$–EtOAc (5:1)] to give the diethyl ester of diacid **14** [diethyl (*E*,*E*)-4,4′-dihydroxy-3,5′-dimethoxy-β,3′-bicinnamate] (76.4 mg, 91.5%) as a yellow syrup, δ_H* 1.21 and 1.24 (3 H each, t, *J* 7.1, 2 × MeCH$_2$O), 3.44 (3 H, s, A3-OMe), 3.96 (3 H, s, B3-OMe), 4.16 and 4.17 (2 H each, q, *J* 7.1, 2 × MeC*H*$_2$O), 6.38 (1 H, d, *J* 15.9, B8-H), 6.696 (1 H, d, *J* 2.1, A2-H), 6.702 (1 H, d, *J* 8.2, A5-H), 6.83 (1 H, dd, *J* 8.2 and 2.1, A6-H), 7.00 (1 H, d, *J* 2.0, B6-H), 7.38 (1 H, d, *J* 2.0, B2-H), 7.56 (1 H, d, *J* 15.9, B7-H) and 7.76 (1 H, s, A7-H); δ_C 14.60 (2 × *Me*CH$_2$O), 55.51 (A3-OMe), 56.58 (B3-OMe), 60.47 and 61.05 (2 × MeC*H*$_2$O), 110.26 (B2), 113.28 (A2), 115.64 (A5), 116.31 (B8), 124.94 (B5), 125.70 (B6), 126.36 (A6), 126.58 (A8), 127.30 (B1), 127.58 (A1), 141.36 (A7), 145.26 (B7), 147.88 (A3), 148.06 (B4), 149.00 (A4), 149.12 (B3), 167.30 (B9) and 167.78 (A9).

Saponification was carried out at room temperature with 40% aq. KOH (5 cm^3). After 2 h, the solution was acidified with 2 mol dm^{-3} HCl, partitioned between EtOAc and saturated aq. NaCl, and the organic layer was dried over Na_2SO_4 and evaporated to afford quantitatively diacid (*E*,*E*)-**14**.

Synthesis of the 8–*O*–4-*Coupled Dehydrodiferulic Acid* **15**.—The synthesis of this 8–O–4-coupled dehydrodimer of ferulic acid is based on Nakatsubo and Higuchi's synthesis of guaiacylglycerol-β-coniferaldehyde ether,[50] Scheme 3. Conversion of coniferaldehyde **24** (Aldrich) into the phenoxyacetate **25** was in 95% yield; acetalization to compound **26** was in 81% yield.

Synthesis of β-*hydroxy ester* **28**. Butyllithium (444 mm^3 of a 2.5 mol dm^{-3} solution, 1.11 mmol) in hexanes was added dropwise to a stirred solution of diisopropylamine (117 mg, 1.16 mmol) in anhydrous THF (4 cm^3) at 0 °C under N_2. Stirring of the mixture was continued for 30 min, after which time the lithium diisopropylamide (LDA) solution was cooled to −78 °C. Compound **26** (285 mg, 0.92 mmol) was dissolved in anhydrous THF (4 cm^3) and added dropwise to the LDA solution. The reaction mixture became brown-yellow and was stirred for 30 min, and 4-*O*-(ethoxyethyl)vanillin **23**[34,73] (207 mg, 0.92 mg) in anhydrous THF (4 cm^3) was then added dropwise to the reaction mixture, which gradually became pale yellow. The reaction mixture was allowed to warm up to room temperature over a period of 1 h. Following quenching by the addition of solid CO_2, the solution was diluted in EtOAc (50 cm^3), washed with saturated aq. NaCl, dried over Na_2SO_4 and evaporated to give a yellow oil (506 mg). Purification by silica gel chromatography [EtOAc–light petroleum (3:2)] yielded the β-hydroxy ester **27** as a 3:1 *erythro*:*threo* mixture (yellow foam, 270 mg, 55%), ^{1}H NMR (selected data; $CDCl_3$ with 0.03% v/v $SiMe_4$) δ_H* 1.47 (3 H, d, *J* 5.1, *Me*CHO$_2$), 4.56 (1 H, d, *J* 6.6, *threo*-A8-H), 4.72 (1 H, d, *J* 5.4, *erythro*-A8-H), 5.06 (1 H, br d, *J* 6.3, *threo*-A7-H), 5.13 (1 H, br t, *erythro*-A7-H), 5.32 (1 H, q, *J* 5.2, MeC*H*O$_2$) 5.36 (1 H, d, *J* 6.0, B9-H), 6.03 (1 H, dd, *J* 15.9 and 6.0, B8-H) and 6.66 (1 H, d, *J* 15.9, B7-H).

Compound **27** (270 mg, 0.51 mmol) was dissolved in THF (5 cm^3) and hydrolysed with 1 mol dm^{-3} HCl (5 cm^3) while the solution was stirred at room temperature for 30 min, after which time the mixture was extracted into EtOAc. The organic layer was washed with saturated aq. NaCl, dried over Na_2SO_4, and evaporated to afford compound **28** as a yellow foam in quantitative yield (212 mg); δ_H*($CDCl_3$ with 0.03% v/v $SiMe_4$): *threo* 1.08 (3 H, t, *J* 7.2, *Me*CH$_2$O), 3.85 and 3.87 (3 H each, s, 2 × MeO), 4.04–4.11 (2 H, m, MeC*H*$_2$O), 4.67 (1 H, d, *J* 6.2, A8-H), 5.10 (1 H, d, *J* 6.2, A7-H), 6.10 (1 H, s, A4-OH), 6.59 (1 H, dd, *J* 15.8 and 7.7, B8-H), 6.78–7.10 (ArH), 7.38 (1 H, d, *J* 15.9, B7-H) and 9.62 (1 H, d, *J* 7.7, B9-H); *erythro* 1.16 (3 H, t, *J* 7.2, MeCH$_2$O), 3.85 and 3.86 (3 H each, s, 2 × MeO), 4.15 (2 H, q, *J* 7.1, MeC*H*$_2$O), 4.81 (1 H, d, *J* 5.4, A8-H), 5.16 (1 H, d, *J* 5.4, A7-H), 6.07 (1 H, s, A4-OH), 6.58 (1 H, dd, *J* 15.8 and 7.7,

B8-H), 6.78–7.10 (ArH), 7.37 (1 H, d, *J* 15.9, B7-H) and 9.61 (1 H, d, *J* 7.7, B9-H).

Ethyl (Z)-β-{4-[(E)-2-*formylvinyl*]-2-*methoxyphenoxy*}-4-*hydroxy*-3-*methoxycinnamate* (Z,E)-**30**. Trimethylsilyl bromide (75.4 mg, 0.493 mmol) was added to a stirred solution of **28** (*erythro*:*threo* mixture; 102.6 mg, 0.246 mmol) in CH_2Cl_2 (5 cm^3). After the mixture had been stirred for 1 h, DBU (112.35 mg, 0.738 mmol) was added. The mixture was stirred for 1 h, after which time it was processed as described for the diethyl ester of compound **14**. The resulting rust-red syrup was then purified by silica gel chromatography [EtOAc–light petroleum (3:2)] to afford a single geometrical isomer (*Z*,*E*)-**30** as a yellow oil (74.2 mg, 76%); δ_H* 1.21 (3 H, t, *J* 7.1, *Me*CH_2O), 3.73 (3 H, s, A3-OMe), 4.00 (3 H, s, B3-OMe), 4.21 (2 H, q, *J* 7.1, MeCH_2O), 6.70 (1 H, dd, *J* 15.9 and 7.7, B8-H), 6.81 (1 H, d, *J* 8.3, A5-H), 6.83 (1 H, d, *J* 8.3, B5-H), 7.18 (1 H, dd, *J* 8.3 and 2.0, B6-H), 7.23 (1 H, dd, *J* 8.3 and 2.0, A6-H), 7.38 (1 H, s, A7-H), 7.49 (1 H, d, *J* 2.0, A2-H), 7.50 (1 H, d, *J* 2.0, B2-H), 7.58 (1 H, d, *J* 15.9, B7-H), 8.14 (1 H, s, A4-OH) and 9.65 (1 H, d, *J* 7.7, B9-H); δ_C 14.48 (*Me*CH_2O), 55.94 (A3-OMe), 56.55 (B3-OMe), 61.76 (MeCH_2O), 112.58 (B2), 113.81 (A2), 114.66 (B5), 116.03 (A5), 123.76 (B6), 125.16 (A1), 126.08 (A6), 128.16 (A7), 128.29 (B8), 130.13 (B1), 138.31 (A8), 148.34 (A3), 149.53 (A4), 149.58 (B4), 150.38 (B3), 153.17 (B7), 163.70 (A9) and 193.89 (B9).

Ethyl (Z)-4-*hydroxy*-3-*methoxy*-β-{2-*methoxy*-4-[(E)-2-*methoxycarbonylvinyl*]*phenoxy*}*cinnamate* (Z,E)-**31**. Compound **30** (64.3 mg, 0.161 mmol) was dissolved in MeOH–CH_2Cl_2 (4:1 v/v, 5 cm^3) and sodium cyanide (40 mg, 0.816 mmol) was added. The mixture was stirred for 15 min at room temperature and gradually turned from yellow to bright orange, indicating formation of the cyanohydrin intermediate. The reaction mixture was then cooled to 0 °C, and manganese dioxide (350 mg, 4.025 mmol) was added. TLC [$CHCl_3$–EtOAc (10:1)] indicated that the clean conversion into the methyl ester **31** was complete after 30 min. After filtration and evaporation, the residue was partitioned between EtOAc and water. The organic layer was washed with saturated aq. NaCl, dried ($MgSO_4$), and evaporated to afford pure diester (*Z*,*E*)-**31** as a yellow oil (65.8 mg, 95%); δ_H* 1.21 (3 H, t, *J* 7.1, *Me*CH_2O), 3.71 (3 H, s, MeOCO), 3.73 (3 H, s, A3-OMe), 4.00 (3 H, s, B3-OMe), 4.17 (2 H, q, *J* 7.1, MeCH_2O), 6.45 (1 H, d, *J* 15.9, B8-H), 6.79 (1 H, d, *J* 8.3, B5-H), 6.81 (1 H, d, *J* 8.3, A5-H), 7.12 (1 H, dd, *J* 8.3 and 2.0, B6-H), 7.22 (1 H, dd, *J* 8.3 and 2.0, A6-H), 7.37 (1 H, s, A7-H), 7.44 (1 H, d, *J* 2.0, B2-H), 7.49 (1 H, d, *J* 2.0, A2-H), 7.59 (1 H, d, *J* 15.9, B7-H) and 8.12 (1 H, s, A4-OH); δ_C 14.47 (*Me*CH_2O), 51.58 (*Me*OCO), 55.92 (A3-OMe), 56.49 (B3-OMe), 61.72 (MeCH_2O), 112.45 (B2), 113.78 (A2), 114.53 (B5), 115.99 (A5), 117.10 (B8), 122.92 (B6), 125.19 (A1), 126.03 (A6), 128.05 (A7), 130.09 (B1), 138.39 (A8), 145.02 (B7), 148.29 (A3), 148.89 (B4), 149.48 (A4), 150.24 (B3), 163.75 (A9) and 167.63 (B9).

(Z)-β-{4-[(E)-2-*Carboxyvinyl*]-2-*methoxyphenoxy*}-4-*hydroxy*-3-*methoxycinnamic acid* (Z,E)-**15**. Compound **31** (60 mg, 0.139 mmol) was dissolved in 1,4-dioxane (1 cm^3) and hydrolysed with 2 mol dm^{-3} NaOH (3 cm^3) at room temperature for 3 h. The reaction mixture was then processed as described for compound **14** to afford diacid (*Z*,*E*)-**15** in quantitative yield as a pale yellow solid, which was recrystallized from acetone–CCl_4, m.p. 208–210 °C; δ_H* 3.73 (3 H, s, A3-OMe), 4.00 (3 H, s, B3-OMe), 6.43 (1 H, d, *J* 15.9, B8-H), 6.82 (1 H, d, *J* 8.2, A5-H), 6.83 (1 H, d, *J* 8.3, B5-H), 7.13 (1 H, dd, *J* 8.3 and 2.0, B6-H), 7.23 (1 H, dd, *J* 8.2 and 2.0, A6-H), 7.42 (1 H, s, A7-H), 7.44 (1 H, d, *J* 2.0, B2-H), 7.52 (1 H, d, *J* 2.0, A2-H), 7.59 (1 H, d, *J* 16.0, B7-H) and 8.12 (1 H, s, A4-OH); δ_C see Table 2. Trimethylsilyl-**15** [Found: M^+, 602.2155 (84%). $C_{29}H_{42}O_8Si_3$ requires M, 602.2188].

Synthesis of the 4–O–5-Coupled Dehydrodiferulic Acid **17** (*Scheme* 4).—*Silver*(I) *oxide oxidation of vanillyl alcohol* **32**. Vanillyl alcohol **32** (154 mg, 1.0 mmol) was dissolved in dry acetone (5 cm^3). Silver(I) oxide (347 mg, 1.5 mmol) was added and the reaction was closely monitored by TLC [$CHCl_3$–EtOAc (1:1)], which indicated disappearance of vanillyl alcohol **32** after typically 30–45 min. The reaction mixture was filtered through Celite and the resulting greenish filtrate was evaporated to give a brown syrup. This product mixture was separated by PLC [$CHCl_3$–EtOAc (1:1), developed three times] to give vanillin (20 mg, 13%), traces of 4–O–5-coupled dehydrodivanillin **34**, unchanged vanillyl alcohol **32** (8 mg, 5%), the mixed 4–O–5 compound **33** (46 mg, 30%) and traces of the 4–O–5-coupled trimer **36**. The remaining oligo/polymeric materials were not investigated further. The use of 1.5–2 cm^3 of acetone per 50 mg of vanillyl alcohol was found to be the best. Although the amount of 4–O–5-coupled dehydrodivanillin was higher when using less dilution, the overall yield of 4–O–5-coupled dehydrodimers did not exceed 20%.

4-Hydroxy-3-(4-hydroxymethyl-2-methoxyphenoxy)-5-methoxybenzaldehyde **33** (yellow oil); δ_H† 3.79 (3 H, s, B3-OMe), 3.95 (3 H, s, A3-OMe), 4.63 (2 H, br s, B1-CH_2OH), 6.91–7.28 (5 H, ArH) and 9.71 (1 H, s, A1-HCO); δ_C 56.19 (B3-OMe), 56.73 (A3-OMe), 64.34 (B1-CH_2OH), 107.98 (A2), 112.52 (B2), 112.62 (A6), 119.78 (B6), 121.17 (B5), 129.00 (A1), 140.84 (B1), 143.92 (A4), 144.30 (B4), 146.98 (A5), 149.86 (A3), 152.00 (B3) and 190.91 (A1-HCO).

3-[4-Formyl-2-(4-hydroxymethyl-2-methoxyphenoxy)-6-methoxyphenoxy]-4-hydroxy-5-methoxybenzaldehyde **36** (brown oil); δ_H† 3.72 (3 H, s, C3-OMe), 3.90 (3 H, s, B3-OMe), 3.94 (3 H, s, A3-OMe), 4.62 (2 H, br s, C1-CH_2OH), 6.88 (1 H, d, *J* 1.7, B6-H), 6.94 (1 H, dd, *J* 8.1 and 1.7, C6-H), 7.00 (1 H, d, *J* 8.1, C5-H), 7.01 (1 H, d, *J* 1.7, A6-H), 7.14 (1 H, d, *J* 1.7, C2-H), 7.26 (1 H, d, *J* 1.7, A2-H), 7.40 (1 H, d, *J* 1.7, B2-H), 9.74 (1 H, s, A1-HCO) and 9.86 (1 H, s, B1-HCO); δ_C 56.07 (C3-OMe), 56.75 (A3-OMe), 56.89 (B3-OMe), 64.25 (C1-CH_2OH), 108.03 (B2), 108.40 (A2), 109.92 (B6), 110.17 (A6), 112.36 (C2), 119.76 (C6), 122.66 (C5), 128.73 (A1), 134.75 (B1), 138.25 (B4), 142.09 (C1), 142.49 (C4), 143.04 (A4), 147.16 (A5), 149.70 (A3), 152.34 (C3), 153.71 (B5), 155.04 (B3), 191.02 (A1-HCO) and 191.69 (B1-HCO).

3-(4-*Formyl*-2-*methoxyphenoxy*)-4-*hydroxy*-5-*methoxybenzaldehyde* **34** *by DDQ oxidation of compound* **33**. DDQ (70 mg, 0.31 mmol, recrystallized from $CHCl_3$) was added to a solution of the primary alcohol **33** (91 mg, 0.30 mmol) in anhydrous THF (5 cm^3). The reaction mixture was stirred under N_2 at room temperature overnight. The solvent was removed by evaporation and the residue was submitted to silica gel chromatography [EtOAc–light petroleum (3:2), then EtOAc] to afford the 4–O–5-coupled dehydrodivanillin **34** (70 mg, 77%) as an oil, which was crystallized from EtOAc–light petroleum, m.p. 135.9–136.8 °C; δ_H† 3.95 (3 H, s, B3-OMe), 3.98 (3 H, s, A3-OMe), 6.94 (1 H, d, *J* 8.2, B5-H), 7.22 (1 H, d, *J* 1.8, A6-H), 7.40 (1 H, d, *J* 1.8, A2-H), 7.48 (1 H, dd, *J* 8.2 and 2.0, B6-H), 7.58 (1 H, d, *J* 2.0, B2-H), 9.80 (1 H, s, A1-HCO) and 9.92 (1 H, s, B1-HCO); δ_C 56.41 (B3-OMe), 56.80 (A3-OMe), 108.48 (A2), 112.26 (B2), 116.99 (A6), 117.63 (B5), 125.55 (B6), 129.54 (A1), 133.56 (B1), 143.79 (A5), 145.22 (A4), 150.37 (A3), 151.49 (B3), 152.48 (B4), 190.75 (A1-HCO) and 191.43 (B1-HCO).

Phenol protection of the 4–*O*–5-*coupled dehydrodivanillin* **34**. Protection of the phenol **34** (50 mg, 0.165 mmol) as the ethoxyethyl derivative as described previously[34,73] afforded compound **35** (52 mg, 84%) as a yellow oil; δ_H† 1.08 (3 H, t, *J* 7.1, *Me*CH_2O), 1.38 (3 H, d, *J* 5.1, *Me*CHO_2), 3.56–3.89 (2 H, non-equivalent, m, MeCH_2O), 3.95 and 3.99 (3 H each, s, 2 × MeO), 5.53 (1 H, q, *J* 5.1, MeCHO_2), 7.04 (1 H, d, *J* 8.1,

† Locants follow the numbering scheme of the parent vanillin framework.

B5-H), 7.11 (1 H, d, *J* 1.8, A6-H), 7.42 (1 H, d, *J* 1.8, A2-H), 7.53 (1 H, dd, *J* 8.1 and 1.9, B6-H), 7.62 (1 H, d, *J* 1.9, B2-H), 9.85 (1 H, s, A1-HCO) and 9.95 (1 H, s, B1-HCO).

Ethyl (E)-3-{4-[(E)-2-*ethoxycarbonylvinyl*]-2-*methoxyphenoxy*}-4-*hydroxy*-5-*methoxycinnamate* (E,E)-**11** (*R* = *Et*). The procedure was similar to that used previously for ^{13}C-labelled ferulic acid.[34,73] Thus, triethyl phosphonoacetate (61 mg, 0.273 mmol) was added to a suspension of NaH (12.5 mg, 0.52 mmol) in dry THF (4 cm^3). After stirring of the mixture for 10 min, a solution of compound **35** (48.7 mg, 0.130 mmol) in THF (4 cm^3 total, with washings) was added. The reaction was quenched after 1 h by addition of 2 mol dm^{-3} HCl, which served also to hydrolyse the ethoxyethyl protecting group. The product was transferred to a separatory funnel with EtOAc and washed twice with aq. NH_4Cl. Drying over $MgSO_4$ and evaporation of the solvent gave the diester **11** (R = Et) as a pale yellow oil which, following NMR spectroscopy, was directly saponified (see below). If the neutralization/deprotection step using HCl was carried on for too long, some de-esterification also occurred but this is not a problem if the subsequent saponification step is to be performed directly.

Compound **11** (R = Et): δ_H* 1.24 (3 H, t, *J* 7.1, A9-OCH_2*Me*), 1.28 (3 H, t, *J* 7.1, B9-OCH_2*Me*), 3.39 (3 H, s, B3-OMe), 3.96 (3 H, s, A3-OMe), 4.16 (2 H, q, *J* 7.1, A9-OCH_2Me), 4.19 (2 H, q, *J* 7.1, B9-OCH_2Me), 6.36 (1 H, d, *J* 15.9, A8-H), 6.48 (1 H, d, *J* 16.0, B8-H), 6.81 (1 H, d, *J* 8.3, B5-H), 6.89 (1 H, d, *J* 1.9, A6-H), 7.17 (1 H, dd, *J* 8.3 and 2.0, B6-H), 7.22 (1 H, d, *J* 1.9, A2-H), 7.46 (1 H, d, *J* 2.0, B2-H), 7.53 (1 H, d, *J* 15.9, A7-H) and 7.62 (1 H, d, *J* 16.0, B7-H); δ_C 14.60 (A9-OCH_2*Me*), 14.60 (B9-OCH_2*Me*), 56.41 (B3-OMe), 56.77 (A3-OMe), 60.53 (A9-OCH_2Me), 60.64 (B9-OCH_2Me), 107.99 (A2), 112.66 (B2), 114.45 (A6), 116.99 (A8), 117.93 (B8), 118.36 (B5), 122.84 (B6), 126.67 (A1), 131.04 (B1), 141.41 (A4), 144.45 (A5), 144.81 (B7), 144.94 (A7), 149.35 (B4), 150.12 (A3), 151.41 (B3), 167.15 (B9) and 167.16 (A9).

(E)-3-{4-[(E)-2-*Carboxyvinyl*]-2-*methoxyphenoxy*}-4-*hydroxy*-5-*methoxycinnamic acid* (E,E)-**17**. Saponification of compound **11** (R = Et) with degassed 50:50 2 mol dm^{-3} NaOH–1,4-dioxane overnight followed by acidification with 2 mol dm^{-3} HCl, extraction into EtOAc, drying over $MgSO_4$, and evaporation gave crude diacid **17**. Silica gel chromatography [$CHCl_3$–EtOAc–HOAc (50:50:1)] gave pure diacid (*E,E*)-**17** which crystallized spontaneously (40 mg, 80% yield over the two steps from dialdehyde **35**, the protected 4–O–5-coupled dehydrodivanillin). Recrystallization from acetone–toluene gave spherulites, m.p. 212.0–214.5 °C (decomp.); δ_H* 3.58 (3 H, s, B3-OMe), 3.88 (3 H, s, A3-OMe), 6.36 (1 H, d, *J* 15.9, A8-H), 6.47 (1 H, d, *J* 15.95, B8-H), 6.82 (1 H, d, *J* 8.3, B5-H), 6.90 (1 H, d, *J* 1.9, A6-H), 7.18 (1 H, dd, *J* 8.3 and 2.0, B6-H), 7.22 (1 H, d, *J* 1.9, A2-H), 7.45 (1 H, d, *J* 2.0, B2-H), 7.54 (1 H, d, *J* 15.9, A7-H) and 7.63 (1 H, d, *J* 15.95, B7-H); δ_C see Table 2. Trimethylsilyl-**17** [Found: M^+, 602.2195 (94%). $C_{29}H_{42}O_8Si_3$ requires M, 602.2188].

Synthesis of the 8–8-*Coupled Dehydrodiferulic Acids* **38**, **18** *and* **19** (*Scheme* 5).—trans-4-(4-*Hydroxy*-3-*methoxybenzylidene*)-2-(4-*hydroxy*-3-*methoxyphenyl*)-5-*oxotetrahydrofuran*-3-*carboxylic acid* **38** [**12b** (*R* = *H*)]. The dilactone **37** (354 mg, 0.917 mmol), prepared according to Cartwright and Haworth's procedure,[56] was dissolved in 2 mol dm^{-3} NaOH (35 cm^3). After being stirred overnight at room temperature under N_2, the reaction mixture was processed as described for compound **14** to give an amber oil, which was submitted to silica gel chromatography [EtOAc–AcOH (100:1)] affording *trans*-lactone acid **38** as an amorphous beige solid (342 mg, 97%); δ_H* 3.81 (3 H, s, A3-OMe), 3.87 (3 H, s, B3-OMe), 4.30 (1 H, t, *J* 2.5, A8-H), 5.75 (1 H, d, *J* 2.8, A7-H), 6.82 (2 H, m, AB part of ABX pattern, A5- and A6-H), 6.89 (1 H, d, *J* 8.2, B5-H), 6.98 (1 H, br s, X part of ABX pattern, A2-H), 7.21 (1 H, dd, *J* 8.2 and 2.0, B6-H), 7.37 (1 H, d, *J* 2.0, B2-H) and 7.61 (1 H, d, *J* 2.1, B7-H); δ_C 54.03 (A8), 56.27 (A3-OMe and B3-OMe), 81.24 (A7), 110.17 (A2), 113.95 (B2), 116.04 (A5), 116.20 (B5), 119.17 (A6), 120.45 (B8), 126.54 (B1), 126.58 (B6), 132.37 (A1), 140.46 (B7), 147.97 (A4), 148.54 (B3), 148.63 (A3), 150.20 (B4), 171.66 (B9) and 172.12 (A9). Traces of the aryldihydronaphthalene **19** were also observed. Trimethylsilyl-**38** [Found: M^+, 602.1596 (0.2%). $C_{29}H_{42}O_8Si_3$ requires M, 602.2188].

Methyl trans-4-(4-*hydroxy*-3-*methoxybenzylidene*)-2-(4-*hydroxy*-3-*methoxyphenyl*)-5-*oxotetrahydrofuran*-3-*carboxylate* **39**. Diazo(trimethylsilyl)methane (Me_3SiCHN_2) (1.608 cm^3 of a 2 mol dm^{-3} solution in portions of 0.268 cm^3, 3.216 mmol) in hexanes was added at room temperature to a solution of the acid **38** (104 mg, 0.269 mmol) in MeOH (10 cm^3) until TLC monitoring [EtOAc–AcOH (100:1) and $CHCl_3$–EtOAc (10:1)] indicated clean and complete conversion. Addition of the Me_3SiCHN_2 in a single aliquot resulted in phenol methylation of up to 20%. Direct evaporation of the reaction mixture yielded the methyl ester *trans*-**39** (97 mg, 90%) as a yellow syrup; δ_H* 3.73 (3 H, s, A9-OMe), 3.81 (3 H, s, A3-OMe), 3.88 (3 H, s, B3-OMe), 4.39 (1 H, t, *J* 2.6, A8-H), 5.72 (1 H, d, *J* 3.0, A7-H), 6.79–6.85 (2 H, m, AB part of ABX pattern, A5- and A6-H), 6.90 (1 H, d, *J* 8.2, B5-H), 6.97 (1 H, br d, X part of ABX pattern, A2-H), 7.17 (1 H, dd, *J* 8.2 and 2.0, B6-H), 7.28 (1 H, d, *J* 2.0, B2-H) and 7.62 (1 H, d, *J* 2.1, B7-H); δ_C 53.16 (A9-OMe), 53.78 (A8), 56.23 and 56.26 (A3-OMe and B3-OMe), 80.97 (A7), 110.12 (A2), 113.78 (B2), 116.04 (A5), 116.24 (B5), 119.23 (A6), 120.03 (B8), 126.38 (B1), 126.42 (B6), 132.08 (A1), 140.75 (B7), 147.99 (A4), 148.52 (B3), 148.61 (A3), 150.24 (B4), 171.45 (B9) and 171.56 (A9).

4,4′-*Dihydroxy*-3,3′-*dimethoxy*-β,β′-*bicinnamic acid monomethyl ester* **40**. The γ-lactone **39** (97 mg, 0.242 mmol) was dissolved in CH_2Cl_2 (10 cm^3) and DBU (150 mm^3, 1.003 mmol) was added. The reaction mixture was stirred at room temperature for 4 h and processed as described for the diethyl ester of compound **14**. Silica gel chromatography [$CHCl_3$–EtOAc–AcOH (1:1:0.1)] afforded compound **40** (87 mg, 90%) as a yellow foam; δ_H* 3.66 (3 H, s, A9-OMe), 3.72 (3 H, s, B3-OMe), 3.73 (3 H, s, A3-OMe), 6.78 (2 H, d, *J* 8.2, A5- and B5-H), 7.09 (1 H, dd, *J* 8.2 and 2.0, B6-H), 7.11 (1 H, dd, *J* 8.2 and 2.0, A6-H), 7.25 (1 H, d, *J* 2.0, B2-H), 7.30 (1 H, d, *J* 2.0, A2-H), 7.81 (1 H, s, A7-H) and 7.84 (1 H, s, B7-H); δ_C 52.28 (A9-OMe), 56.04 (A3-OMe and B3-OMe), 113.40 (B2), 113.54 (A2), 115.95 (B5), 115.97 (A5), 125.60 (B6), 125.67 (A8 and B8), 125.73 (A6), 127.78 (B1), 127.81 (A1), 142.36 (A7), 142.47 (B7), 148.19 (A3), 148.20 (B3), 149.32 (B4), 149.37 (A4), 168.22 (A9) and 168.53 (B9).

4,4′-*Dihydroxy*-3,3′-*dimethoxy*-β,β′-*bicinnamic acid* **18**. Compound **40** was saponified as described for compound **14** to afford quantitatively the 8–8-*coupled dehydrodiferulic acid* **18** as a yellow solid, which was recrystallized from aq. MeOH, m.p. 230–236 °C (decomp.); δ_H* 3.74 (6 H, s, 2 × MeO), 6.78 (2 H, d, *J* 8.2, A5-H), 7.11 (2 H, dd, *J* 8.2 and 2.0, A6-H), 7.31 (2 H, d, *J* 2.0, A2-H) and 7.83 (2 H, s, A7-H); δ_C see Table 2. Trimethylsilyl-**18** [Found: M^+, 674.2563 (14%). $C_{32}H_{50}O_8Si_4$ requires M, 674.2583].

trans-7-*Hydroxy*-1-(4-*hydroxy*-3-*methoxyphenyl*)-6-*methoxy*-1,2-*dihydronaphthalene*-2,3-*dicarboxylic acid* trans-**19**. The γ-lactone *trans*-**38** (50 mg, 0.129 mmol) was dissolved in 1,4-dioxane–1 mol dm^{-3} HCl (1:1; 10 cm^3) and the solution was heated under reflux for 3 h. The reaction mixture was cooled, diluted in EtOAc, washed with saturated aq. NaCl, and the organic layer was dried over Na_2SO_4 and evaporated. The residue was submitted to silica gel chromatography [EtOAc–AcOH (100:1)] to afford the 8–8-*coupled dehydrodiferulic acid* trans-**19** (42 mg, 84%) as an off-white solid, m.p. 175–180 °C (decomp.); δ_H* 3.74 (3 H, s, A3-OMe), 3.86 (3 H, s, B3-OMe),

3.88 (1 H, d, *J* 1.8, A8-H), 4.61 (1 H, br d, *J* 1.8, A7-H), 6.42 (1 H, dd, *J* 8.2 and 2.0, A6-H), 6.64 (1 H, d, *J* 8.2, A5-H), 6.71 (1 H, s, B5-H), 6.79 (1 H, d, *J* 2.0, A2-H), 7.04 (1 H, s, B2-H) and 7.60 (1 H, s, B7-H); δ_C see Table 2. Trimethylsilyl-**19** [Found: M^+, 674.2497 (9%). $C_{32}H_{50}O_8Si_4$ requires M, 674.2583].

Appendix

Since submitting this manuscript, two published articles describe the discovery of isomeric dehydrodimers. Van Huystee and Zheng[74] observed six products by TLC and hypothesized that three of them must be the (*E,E*), (*E,Z*) and (*Z,Z*) isomers of the 5–5-coupled dehydrodimer. Stewart *et al.*[75] came to similar conclusions, hypothesizing further orientational isomers, after noting six peaks in their GLC runs that had identical mass spectra. Each of these papers was based on the premise that 5–5-coupling is the only coupling pathway allowable, a misconception revealed by the present study. Photochemical isomerism was tested during the course of the work described in this paper (by irradiation of some of the compounds described here)—only traces of other geometrical isomers could be detected in our samples.[76]

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Mention of trade name, proprietary product, or specific equipment does not constitute a guarantee of the product by USDA and does not imply its approval to the exclusion of other products that may also be suitable.

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